Effects of ruminal and postruminal infusion of starch hydrolysate or glucose on the microbial ecology of the gastrointestinal tract in growing steers

J. S. Van Kessel, P. C. Nedoluha, A. Williams-Campbell, R. L. Baldwin, VI and K. R. McLeod

J Anim Sci 2002. 80:3027-3034.

Effects of ruminal and postruminal infusion of starch hydrolysate or glucose on the microbial ecology of the gastrointestinal tract in growing steers[1,2]

J. S. Van Kessel*[3], P. C. Nedoluha†, A. Williams-Campbell†, R. L. Baldwin VI‡, and K. R. McLeod[4]‡

*Animal Waste Pathogen Laboratory; †Food Safety and Technology Laboratory; and ‡Animal Manure and Byproducts Laboratory, Animal and Natural Resources Institute, USDA-ARS, Beltsville, MD

ABSTRACT: Forty crossbred steers were used to determine the effects of carbohydrate supply site on the indigenous bacteria of the gastrointestinal tract. Steers were fitted with ruminal and abomasal infusion catheters and assigned randomly to one of eight groups in a complete randomized block design. The experimental period was 36 d. Treatments included: 1) a pelleted basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} (LE); 2) the basal diet fed at 0.215 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} (HE); 3) the basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with ruminal infusion of starch hydrolysate (SH) (RSH); 4) the basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with abomasal infusion of SH (ASH); and 5) the basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with abomasal infusion of glucose (AG). The total volume of infusate (5 kg·site^{-1} · d^{-1}) was equalized across treatments and infusion sites by infusion of water. Glucose and SH were infused at rates of 14.35 and 12.64 g · (kg $BW^{0.75}$)·d^{-1}, respectively. Ruminal, cecal, and fecal samples were obtained on d 36. Ruminal pH was low (5.79) in LE steers and unaffected ($P > 0.10$) by increased energy intake or carbohydrate infusion. Cecal and fecal pH were 6.93 and 7.00, respectively, for LE steers. Increasing energy intake ($P < 0.10$) and the rate of carbohydrate infusion ($P < 0.01$) significantly decreased cecal and fecal pH compared with LE. Ruminal counts of anaerobic bacteria in LE steers were 8.99 $\log_{10}$ cells/g and abomasal carbohydrate infusion had no affect ($P > 0.10$) on these numbers. However, ASH and AG steers had approximately 1.5 $\log_{10}$ cells/g more ($P < 0.01$) cecal and fecal anaerobic populations. Ruminal, cecal, and fecal aerobic bacterial counts were 40, 22, and 23%, respectively, lower than anaerobic counts. Generally, aerobic counts responded similarly to the anaerobic counts. Less than 1% of the anaerobic bacteria enumerated in the rumen, cecum, and feces were coliforms, and 97% of the coliforms were *Escherichia coli*. Carbohydrate infusions resulted in only numerical increases in fecal coliform and *E. coli* concentrations ($P > 0.10$). Fecal *E. coli* were highly acid sensitive in all steers, with less than 1% surviving a 1-h exposure to low pH (2.0). This suggests that cecal or fecal pH is not a good indicator of acid resistance, and it supports the concept that there are other factors that may induce acid resistance.

Key Words: Bacteria, Cattle, *Escherichia coli*, Fermentation, Rumen

 J. Anim. Sci. 2002. 80:3027–3034

Introduction

Concern over the transfer of zoonotic pathogens from animal production systems to the food and water supply has increased in recent years. Several outbreaks of *Escherichia coli* O157:H7 infections in humans have been associated with the consumption of beef and dairy products (USDA-APHIS, 1997). Cattle have also been implicated as the source of outbreaks due to contaminated water supplies.

Although postharvest remediation is often effective in reducing pathogen numbers (Elder et al., 2000), a more direct approach is to reduce shedding. Epidemiological studies have demonstrated correlations between *E. coli* O157:H7 shedding by cattle with various management factors (USDA-APHIS, 1997). Thus, changes in management strategies may be an effective means of reducing pathogen shedding.

Accurate prediction of responses to dietary changes for individual microbial species or groups of microbial

[1]Mention of a trade name, vendor, proprietary product, or specific equipment is not a guarantee or a warranty by the USDA, and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

[2]The authors gratefully acknowledge T. Jacobs and C. Mudd for technical assistance.

[3]Correspondence: Bldg. 173, Rm. 204, BARC-East (phone: 301-504-8287; fax: 301-504-6608; E-mail: jkessel@anri.barc.usda.gov).

[4]Current address: Univ. of Kentucky, Dept. Anim. Sci., 806 W. P. Garrigus Bldg., Lexington 40546-0215.

Received December 28, 2001.

Accepted July 16, 2002.

Table 1. Basal diet ingredients and chemical composition[a]

Ingredient	DM, % (SEM)
Orchard grass hay	89.45
Corn gluten meal	5.00
Soypass[b]	5.00
Trace mineral–salt mix	0.50
Vitamins	0.05
Chemical	
CP	19.5 (0.149)
Energy[c]	4571 (4.42)
NDF	47.3 (0.53)
ADF	28.7 (0.32)

[a]There was no difference ($P < 0.01$) in diet composition across treatments.
[b]Lignotech USA, Rothschild, WI.
[c]Energy given in cal/g.

species is difficult. In fact, recent studies assessing dietary composition effects on *E. coli*-shedding patterns of cattle are inconsistent and conflicting (Diez-Gonzalez et al., 1998; Hovde et al., 1999; Tkalcic et al., 2000). Nevertheless, in order to reduce pathogen shedding, an accurate understanding of the growth-promoting or -restricting conditions of the digestive tract must be established.

Based on the hypothesis that nutrient supply to the lower tract impacts pathogen shedding, our long-term objective is to develop a model system for determining conditions under which pathogenic bacteria proliferate or are inhibited in cattle. Current research within our institute is also directed at understanding the differential effects of carbohydrate infusion site on the energetic efficiency of nutrient use in growing beef animals. Thus, we chose to evaluate the direct infusion of carbohydrate as a model system for our experiments. The specific objectives of the current experiment are to determine the effects of carbohydrate supply site on the indigenous bacteria of the gastrointestinal tract of steers.

Materials and Methods

Animals and Experimental Protocol

Forty crossbred beef steers (initial BW was 243.5 ± 2.40 kg) were either obtained from the beef herd at the Beltsville Agricultural Research Center or purchased as uniform groups of 10 from individual producers in the region. The Beltsville Area Animal Care and Animal Use Committee (Protocol # 98-025) approved all procedures involving these steers.

Each steer was surgically fitted with ruminal and abomasal infusion catheters and assigned randomly to one of eight groups (blocks) with each treatment being represented in each group (complete randomized block design with five treatments in eight blocks). Treatments included the following: 1) a pelleted basal diet (Table 1) fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with ruminal and abomasal infusion of water (negative control; LE); 2) the basal diet fed at 0.215 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with ruminal and abomasal infusion of water (positive control; **HE**); 3) the basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with ruminal infusion of starch hydrolysate (**SH**) and abomasal infusion of water (**RSH**); 4) the basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with ruminal infusion of water and abomasal infusion of SH (**ASH**); and 5) the basal diet fed at 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with ruminal infusion of water and abomasal infusion of glucose (**AG**). Diet ME was assumed to be 2.415 Mcal/kg. Infusates were prepared in tap water and composed of a 20% (wt/wt) solution of partially hydrolyzed maize starch (Bauer et al., 2001) or 25% (wt/wt) glucose. Glucose and SH were infused at a rate of 14.4 and 12.6 g·(kg $BW^{0.75}$)$^{-1}$·d^{-1}, respectively, to achieve isocaloric amounts of infusates. Infusion rates were selected based on data that demonstrated starch digestion and absorption capacity is exceeded when more than 800 g of starch is infused to the abomasum daily in growing, starch-adapted steers (Branco et al., 1999).

One week prior to the initiation of the experimental period, steers were adapted to the basal diet at an intake of 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1}. Basal diets were fed in 12 equal portions daily and the total volume of infusate (5 kg·site^{-1}·d^{-1}) was equalized across treatments and infusion sites by infusion of water. Steers were adapted to dietary intake (HE) and SH and glucose infusions over the first 7 d of each 36-d period. The daily amount of feed offered, and SH or glucose infused, was adjusted at the beginning of each week based on BW.

Sampling

Infusions were discontinued on d 36. Fecal grab samples (~500 g) were obtained from approximately 15 to 18 cm distal to the anus. Steers were stunned humanely and exsanguinated by trained abattoir staff, and viscera were removed. Digesta was obtained from the rumen and the lower tract. A slit was made in the side of the rumen and aliquots (~250 mL) of whole rumen contents were removed from each of six approximate locations (lower reticulum, rumen, and ventral sac, and upper reticulum, rumen, and ventral sac) by the same person throughout the study. Approximately 1,000 mL of contents was also collected from the cecum.

All digesta samples were collected into sterile containers, mixed, and separated into appropriate aliquots for microbial analyses. The pH (model 350; Orion Research, Inc., Boston, MA) of each sample was determined prior to separation, within 15 min of collection. Ruminal and cecal content pH measurements were done by immersing the probe directly into the sample, but because of the low moisture content in the feces, fecal samples (25 g) were diluted with 50 mL sterile peptone water (0.1%; Difco, Sparks, MD) to facilitate pH analysis.

Microbiological Assays

Total anaerobic bacterial concentrations were estimated by a three-tube most probable number procedure using Bryant and Robinson's (1961) nonselective anaerobic medium. Approximately 25 g of whole ruminal, cecal, or fecal contents was weighed into sterile 400-mL filter bags (Microbiology International, Frederick, MD), which were continuously purged with O_2-free CO_2 and diluted 10-fold with the enumeration medium. Diluted samples were homogenized for 2 min in a Bagmixer (Interscience, St. Nom, France). Homogenates were serially diluted (1:10) in roll tubes to 10^{-12}, and then transferred to each of three tubes. Tubes were incubated at 39°C for 14 d and were scored as positive for growth based on the presence of turbidity.

The aerobic microbiological analyses were performed in triplicate. Ruminal (10 g), cecal (25 g), and fecal (25 g) samples were weighed into sterile 400-mL filter bags and diluted 10-fold with sterile 0.1% peptone water using an automatic diluter (Dilumat 3 mk2; Combourg, France). Fecal samples were homogenized for 2 min in a Bagmixer; ruminal and cecal samples were homogenized for 2 min on high speed in a Stomacher 400 Lab Blender (Seward Medical, London, UK). Homogenates were serially diluted in 0.1% peptone water and spiral plated in duplicate onto plate-count agar (**PCA;** Difco) or MacConkey agar with 0.1% 4-methyllumbelliferyl-β-D-glucuronide (**MAC-MUG;** Criterion, Hardy Diagnostics; Santa Maria, CA). Spiral plating of fecal samples was conducted with a WASP 2 spiral plater (Don Whitley Scientific Limited, West Yorkshire, UK), and the ruminal and cecal samples were plated with an Autoplate 4000 (Spiral Biotech, Norwood, MA). PCA plates were incubated for either 24 h (fecal, cecal) or 48 h (ruminal) at 37°C. MAC-MUG plates were incubated at 37°C for 18 to 24 h. Red or pink colonies were considered positive for coliforms, and colonies that were red or pink and fluorescent (365 nm) were considered positive for *E. coli*.

The acid shock procedure was conducted in duplicate on fecal samples only. Five mL of the 10^{-1} dilution (see above) was transferred to a bottle containing 45 mL of pH 2.0 acid shock medium (Diez-Gonzalez and Russell, 1999) and incubated at 37°C for 1 h. *E. coli* were enumerated in the acid-shocked and nonacid-shocked samples by MPN using Fluorocult LMX broth (EM Science; Gibbstown, NJ). This is a selective enrichment broth for the simultaneous detection of total coliforms and *E. coli*. It contains a chromogenic substrate (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) that is cleaved by coliforms. Fluorocult LMX broth also contains 4-methyllumbelliferyl-β-D-glucuronide—which is cleaved by *E. coli,* but not other coliforms—yielding a product that can be detected under UV light. After the incubation, the acid-shocked samples were serially diluted with sterile 0.1% peptone water. Serially diluted samples were inoculated into Fluorocult LMX broth tubes (0.5 mL into 4.5 mL) and the tubes were incubated at 37°C for 24 h. Tubes were scored positive according to the manufacturer's directions. Briefly, tubes that remained yellow were scored negative. Blue-green colored tubes (coliform-positive) were checked for fluorescence (365 nm), and Kovac's reagent (5% *p*-dimethylaminobenzaldehyde in 3:1 butanol:HCl [vol/vol]) was added to all positive tubes (Indole reaction). Positive indole reactions confirmed the presence of viable *E. coli*.

Statistical Analyses

All statistical analyses were conducted using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The experimental design was a randomized complete block with the five treatments as the main effects and the infusion group as the block effect. ANOVA for measured variables was performed using group, treatment, and interaction of group × treatment in the model. Effects of dietary intake (LE vs HE), starch infusion (LE vs RSH and ASH), ruminal starch infusion (LE vs RSH), abomasal starch infusion (LE vs ASH), site of starch infusion (RSH vs ASH), and glucose infusion (LE vs AG) were tested using nonorthogonal contrast (Snedecor and Cochran, 1980). One steer in the ASH treatment group was removed from the study on the last day of infusion due to injury. Some samples were lost during analysis and this is reflected in the reported number of observations. All data are presented as arithmetic means with maximal standard error reported for each sampling location (ruminal, cecal, fecal).

Results

Ruminal, cecal, and fecal pH levels were 5.79, 6.93, and 7.00, respectively, when steers were fed the basal diet (Table 2). Although ruminal pH was not different ($P > 0.10$) for steers fed the HE diet vs the LE diet, the HE diet resulted in somewhat lowered ($P < 0.10$) cecal and fecal pH (6.93 vs 6.58 and 7.00 vs 6.77, respectively). Infusion of SH or glucose had no impact ($P > 0.10$) on ruminal pH, regardless of the infusion site. Cecal pH tended to be lower for RSH ($P < 0.10$), and was lower for ASH ($P < 0.01$) and AG ($P < 0.01$) than for LE animals. Fecal pH followed a similar pattern. The observed differences in pH were greater ($P < 0.01$) when SH was infused into the abomasum vs the rumen.

When steers were fed the LE or HE diets, there was no difference ($P > 0.10$) in total anaerobic bacterial concentrations in the rumen, cecum, or feces (Table 3). The infusion of SH into the rumen resulted in a slightly lower ($P < 0.10$) total anaerobic count in the rumen, but had no effect ($P > 0.10$) on cecal or fecal anaerobic numbers. Steers that were abomasally infused with SH or glucose had at least 1.3 $\log_{10}$ colony-forming units (CFU) per gram more ($P < 0.01$) total anaerobic bacteria in both the cecum and feces than LE steers.

Plate-count agar is a nonselective medium commonly used to enumerate aerobic bacteria in many matrices, including wastewater (Difco Manual, Difco Labora-

Table 2. pH of ruminal contents, cecal contents, and feces of steers fed two levels of dietary energy and infused ruminally with starch hydrolysate or abomasally with starch hydrolysate or glucose (n = 8)

Location	LE	HE	RSH	ASH	AG	SE	I[a,b]	SH[a,b]	RSH[a,b]	ASH[a,b]	R vs A[a,b]	AG[a,b]
Rumen	5.79	5.68	5.63	5.80[c]	5.63[c]	0.18	NS	NS	NS	NS	NS	NS
Cecum	6.93	6.58	6.60	5.58[c]	6.38	0.24	†	**	†	**	**	**
Feces	7.00	6.77	6.63	5.22[c]	6.54	0.15	†	**	**	**	**	**

[a]Low intake (LE) with H_2O infusion vs high intake (HE) with H_2O infusion (I), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion and low intake with abomasal starch hydrolysate infusion (SH), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion (RSH), low intake with H_2O infusion vs low intake with abomasal starch hydrolysate infusion (ASH), low intake with ruminal starch hydrolysate infusion vs low intake with abomasal starch hydrolysate infusion (R vs A), low intake with H_2O infusion vs low intake with abomasal glucose infusion (AG).
[b]Probability of a larger variance ratio statistic; †$P < 0.10$, **$P < 0.01$; NS = not significant.
[c]n = 7.

tories; Sparks, MD). Although, for obvious reasons, PCA is not typically used for bacterial enumerations in anaerobic systems, we were interested in comparing trends in both aerobic and anaerobic bacterial counts. There are numerous facultative anaerobic bacteria in the rumen and lower tract, including food-borne pathogens such as *E. coli* and *Salmonella* spp.

Total bacterial counts from PCA were 5.39, 5.98, and 6.11 $\log_{10}$ CFU/g of ruminal contents, cecal contents, and feces, respectively, in LE steers (Table 4), and these were not different ($P > 0.10$) from those of HE steers. When SH was infused in the rumen, ruminal aerobic counts were 0.88 $\log_{10}$ CFU/g greater ($P < 0.01$) than LE aerobic ruminal counts, but abomasal SH infusion had no effect ($P > 0.10$) on ruminal counts. Both abomasal SH and glucose infusion resulted in more than 1 $\log_{10}$ CFU/g higher cecal ($P < 0.05$ and $P < 0.01$, respectively) and fecal ($P < 0.01$) aerobic counts.

MacConkey is a selective medium for gram-negative bacteria, and it is often used for the isolation of coliforms (Difco Manual; Difco Laboratories). Coliforms, such as *E. coli,* are mostly of cecal origin and, therefore, coliform enumeration is often used as an indicator for the presence of fecal contamination. Total coliform concentrations were 3.12, 5.45, and 5.82 $\log_{10}$ CFU/g in ruminal contents, cecal contents, and feces, respectively, for LE steers (Table 5). When comparing the LE and HE steers, there was no difference ($P > 0.10$) in coliform numbers in any of the sample locations. Similarly, ruminal infusion of SH did not affect ($P > 0.10$) coliform numbers in any location. However, there were more coliforms in the rumen ($P < 0.05$) and there tended to be more in the cecum ($P < 0.10$) of steers infused abomasally with SH. There were no significant differences in coliforms at any location between AG steers and LE steers.

E. coli produces the enzyme β-glucuronidase, which degrades MUG and releases fluorescent 4-methylumbelliferone. MUG-positive colonies fluoresce blue-white under UV light. Therefore, fluorescent colonies on MAC-MUG plates were scored positive for *E. coli.* Most of the colonies that were positive for coliforms on the MAC-MUG plates (pink or red colonies) also fluoresced, and were scored positive for *E. coli* (Table 6). Similar to the coliform count, ASH steers had greater ruminal ($P < 0.05$)—and a tendency toward greater cecal ($P < 0.10$)—*E. coli* populations than the LE steers, and there was no difference ($P > 0.10$) in counts between AG steers and LE steers. None of the infusion treatments had an effect ($P > 0.10$) on fecal *E. coli* numbers.

When fecal samples from the LE steers were subjected to an acid medium (pH 2) for 1 h, there was a 2.84 $\log_{10}$ reduction of *E. coli.* A significantly greater ($P < 0.05$) reduction (3.68 $\log_{10}$) was observed in samples from the HE steers compared with the LE steers. Although numerically greater, the reduction in *E. coli* numbers due to acid-shock in samples from the SH-infused steers was not different ($P > 0.10$) from the

Table 3. Total anaerobic bacterial concentrations ($\log_{10}$ cells/g) in ruminal contents, cecal contents, and feces of steers fed two levels of dietary energy and infused ruminally with starch hydrolysate or abomasally with starch hydrolysate or glucose (n = 8)

Location	LE	HE	RSH	ASH	AG	SE	I[ab]	SH[ab]	RSH[ab]	ASH[ab]	R vs A[ab]	AG[ab]
Rumen	8.99	8.91	8.42	9.08[c]	9.09[c]	0.53	NS	NS	†	NS	**	NS
Cecum	7.65	7.54	7.61	9.06[c]	9.17	0.55	NS	†	NS	**	**	**
Feces	7.92	7.94	7.94	9.32[c]	9.29	0.40	NS	*	NS	**	**	**

[a]Low intake (LE) with H_2O infusion vs high intake (HE) with H_2O infusion (I), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion and low intake with abomasal starch hydrolysate infusion (SH), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion (RSH), low intake with H_2O infusion vs low intake with abomasal starch hydrolysate infusion (ASH), low intake with ruminal starch hydrolysate infusion vs low intake with abomasal starch hydrolysate infusion (R vs A), low intake with H_2O infusion vs low intake with abomasal glucose infusion (AG).
[b]Probability of a larger variance ratio statistic; †$P < 0.10$, *$P < 0.05$, **$P < 0.01$; NS = not significant.
[c]n = 7.

Table 4. Total aerobic bacterial concentrations (log_{10} colony-forming unit per gram) of ruminal contents, cecal contents, and feces of steers fed two levels of dietary energy and infused ruminally with starch hydrolysate or abomasally with starch hydrolysate or glucose (n = 8)

Location	LE	HE	RSH	ASH	AG	SE	I[ab]	SH[ab]	RSH[ab]	ASH[ab]	R vs A[ab]	AG[ab]
Rumen	5.39	5.46	6.27	5.68[c]	5.76[c]	0.12	NS	**	**	NS	**	NS
Cecum	5.98	6.34	6.03	7.21[c]	7.34	0.26	NS	NS	NS	*	*	**
Feces	6.11	6.52	6.24	7.85[c]	7.42	0.27	NS	*	NS	**	**	**

[a]Low intake (LE) with H_2O infusion vs high intake (HE) with H_2O infusion (I), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion and low intake with abomasal starch hydrolysate infusion (SH), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion (RSH), low intake with H_2O infusion vs low intake with abomasal starch hydrolysate infusion (ASH), low intake with ruminal starch hydrolysate infusion vs low intake with abomasal starch hydrolysate infusion (R vs A), low intake with H_2O infusion vs low intake with abomasal glucose infusion (AG).
[b]Probability of a larger variance ratio statistic; *$P < 0.05$, **$P < 0.01$; NS = not significant.
[c]n = 7.

LE samples. Abomasal glucose infusion also had no significant effect ($P > 0.10$) on the resistance of *E. coli* to an acid medium.

Discussion

Bacterial growth is usually sensitive to changes in environmental pH. Each species generally has a defined optimal pH for growth and a pH range where growth is possible. Therefore, slight changes in pH can have dramatic impacts on the ecology of niche populations. Ruminal pH typically ranges from 6.5 to 6.7 in forage-fed animals and decreases as the amount of fermentable carbohydrate in the diet is increased (Van Soest, 1994).

Although the basal diet in the current experiment consisted predominately of orchard grass hay (Table 1), ruminal pH (5.79) in LE steers was indicative of an energy-dense diet (Table 2). The basal diet was pelleted to reduce intake problems, but pelleting also decreases particle size, and subsequently, effective fiber (Mertens, 1983). Therefore, this diet had less ability to support appropriate ruminal mat formation and rumination than its high NDF concentration (47%) would indicate. Increasing the intake of the basal diet (HE) had no significant effect on ruminal pH, although cecal and fecal pH were decreased. A plausible explanation for this is that more of the diet escaped ruminal fermentation and was subsequently degraded and fermented in the small and large intestine. Volatile fatty acids such as acetate, propionate, and butyrate are end products of anaerobic microbial metabolism. The pk's of the VFA are low (4.8 to 4.9) and VFA accumulation in the rumen or intestines results in a drop in pH (Allison, 1984).

The infusion of SH into the rumen had no effect on ruminal pH, but cecal and fecal pH were lower. Either ruminally infused SH reached the small and large intestines or, more likely, ruminal fermentation of SH depressed ruminal fermentation of the basal diet. Thus, passage of fermentable carbohydrate to the large intestine was increased. The optimal pH for cellulose degradation in the rumen is 6.9 to 7.0 (Russell and Wilson, 1996; Weimer, 1996). Therefore, because cellulolytic activity at pH 5.8 is decreased and highly sensitive to even slight drops in pH, fiber degradation was likely compromised. Moreover, the total number of anaerobic bacteria in the rumen was almost fourfold lower in the RSH steers vs the LE steers, suggesting major changes in growth rates or shifts in the bacterial population.

Abomasal SH infusion ensures the availability of starch for digestion, fermentation, and absorption in the lower tract. This was evidenced by large decreases in both cecal and fecal pH for the ASH steers. Based on the observed differences in pH, more SH appeared to get to the large intestine in abomasally infused steers than in the ruminally infused steers. Similarly, abomasal glucose infusion also decreased both cecal and fecal

Table 5. Total coliform concentrations (log_{10} colony-forming units per gram) in ruminal contents, cecal contents, and feces of steers fed two levels of dietary energy intake and infused ruminally with starch hydrolysate or abomasally with starch hydrolysate or glucose (n = 8)

Location	LE	HE	RSH	ASH	AG	SE	I[ab]	SH[ab]	RSH[ab]	ASH[ab]	R vs A[ab]	AG[ab]
Rumen	3.12	3.70	3.41	3.95[c]	3.56[c]	0.25	NS	†	NS	*	NS	NS
Cecum	5.45	5.63	5.84	6.40[c]	6.02	0.33	NS	NS	NS	†	NS	NS
Feces	5.82	5.82	6.00	6.04[c]	6.21	0.29	NS	NS	NS	NS	NS	NS

[a]Low intake (LE) with H_2O infusion vs high intake (HE) with H_2O infusion (I), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion and low intake with abomasal starch hydrolysate infusion (SH), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion (RSH), low intake with H_2O infusion vs low intake with abomasal starch hydrolysate infusion (ASH), low intake with ruminal starch hydrolysate infusion vs low intake with abomasal starch hydrolysate infusion (R vs A), low intake with H_2O infusion vs low intake with abomasal glucose infusion (AG).
[b]Probability of a larger variance ratio statistic; †$P < 0.10$, *$P < 0.05$; NS = not significant.
[c]n = 7.

Table 6. *Escherichia coli* concentrations (log_{10} colony-forming per gram) in ruminal contents, cecal contents, and feces of steers fed two levels of dietary energy intake and infused ruminally with starch hydrolysate or abomasally with starch hydrolysate or glucose (n = 8)

Location	LE	HE	RSH	ASH	AG	SE	I[ab]	SH[ab]	RSH[ab]	ASH[ab]	R vs A[ab]	AG[ab]
Rumen	3.03	3.56	3.24	3.92[c]	3.47[c]	0.28	NS	NS	NS	*	*	NS
Cecum	5.26	5.55	5.72	6.32[c]	5.89	0.35	NS	†	NS	*	NS	NS
Feces	5.67	5.78	5.85	5.89[c]	6.09	0.29	NS	NS	NS	NS	NS	NS

[a]Low intake (LE) with H_2O infusion vs high intake (HE) with H_2O infusion (I), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion and low intake with abomasal starch hydrolysate infusion (SH), low intake with H_2O infusion vs low intake with ruminal starch hydrolysate infusion (RSH), low intake with H_2O infusion vs low intake with abomasal starch hydrolysate infusion (ASH), low intake with ruminal starch hydrolysate infusion vs low intake with abomasal starch hydrolysate infusion (R vs A), low intake with H_2O infusion vs low intake with abomasal glucose infusion (AG).
[b]Probability of a larger variance ratio statistic; $P < 0.10$, $*P < 0.05$; NS = not significant.
[c]n = 7.

pH; however, this was not to the extent observed for the ASH steers. The extent to which nutrients reach the large intestine is affected by both the rate at which starch is hydrolyzed to glucose, as well as the rate of glucose absorption. When the small intestine's capacity to digest and absorb the delivered starch hydrolysate is exceeded, fermentation occurs in the large intestine (Siciliano-Jones and Murphy, 1989; Harmon and McLeod, 2001). Based on this concept, the small intestine's capacity to digest the starch was somewhat exceeded, resulting in greater large intestine fermentation, and subsequently, lower pH.

As expected, abomasal carbohydrate infusion (ASH, AG) had no affect on ruminal anaerobic bacteria numbers. However, the increase in energy supplied postruminally resulted in increases in both cecal and fecal anaerobic populations by 1.3 to 1.5 log_{10} cells/g. Based on the observed low fecal pH (5.22) in the ASH steers, it is likely that these increases in bacterial numbers also reflect a population shift toward more acid-tolerant groups of bacteria.

Total aerobic bacterial counts were 3.60, 1.67, and 1.81 log_{10} CFU/g lower than the anaerobic counts in the rumen, cecum, and feces, respectively (Tables 3 and 4). Facultative anaerobes represent a higher percentage of the bacterial populations in the large intestines than in the rumen (Allison, 1984). With the exception of ruminal response to ruminal SH infusion, aerobic bacterial numbers responded similarly to the anaerobic numbers for each of the infusates at each infusion location. Aerobic counts were higher in the rumen of RSH steers vs LE steers, whereas anaerobic counts were slightly lower. Based on these results, aerobic enumeration may be a useful tool for monitoring bacterial response to changes in dietary conditions, particularly in the small and large intestines.

There is a considerable overlap of predominant indigenous bacterial species between the lower tract and the rumen (Allison, 1984). In the current study, less than 1% of the anaerobes enumerated in the rumen, cecum, and feces were coliforms, and 97% of the coliforms were enumerated as *E. coli* (Tables 5 and 6). Observed concentrations of fecal *E. coli* were somewhat lower in the current study than in reports by Allison et al. (1975) and Diez-Gonzalez et al. (1998), but diets were different in each of the studies. Coliforms, particularly *E. coli,* are typically more prevalent in the lower tract than in the rumen, and this was supported by this data.

The only treatment that elicited a change in coliform and *E. coli* numbers was ASH. Steers that were abomasally infused with SH had higher counts in the rumen and in the cecum. Since there was no change in nutrients entering the rumen, there was no explanation for the higher ruminal counts.

Diez-Gonzalez et al. (1998) demonstrated a decrease in colonic total coliform and *E. coli* concentrations when the diets of cattle were shifted from a concentrate-based to a forage-based diet. In the current study, fecal *E. coli* were numerically higher in the steers infused abomasally with SH or glucose vs the LE steers, but this difference was not statistically significant. There are two possible explanations for the apparently disparate changes observed in coliform and *E. coli* numbers. The change in energy density is less dramatic with the carbohydrate infusions (20% of total metabolizable energy intake) than when making a complete switch from a concentrate to forage diet. Also, corn is a more complex matrix to degrade than SH, and therefore, more hindgut fermentation would occur.

Due to potential differences in physiological traits such as acid resistance, all *E. coli* strains will not necessarily respond similarly to environmental changes, such as decreases in pH. In order for a pathogenic bacterium such as *E. coli* O157:H7 to exhibit its virulence, it must survive the acidic conditions of the gastric stomach. Acid tolerance can be induced in *E. coli* O157:H7 by growing it in acidic conditions, and there have been several recent reports on the acid-resistant properties of colonic *E. coli* (Diez-Gonzalez et al., 1998; Diez-Gonzalez and Russell, 1999; Hovde et al., 1999; Tkalcic et al., 2000).

Diez-Gonzalez et al. (1998) observed that the acid-resistance capabilities of fecal *E. coli* were reduced (approximately 10 vs 0.01% survival) as a result of a dietary switch from concentrate to forage. The authors hypothesized that these differences in response to acid

Table 7. Fecal *Escherichia coli* concentrations (log_{10} colony-forming units per gram) before and after incubation in acid shock medium (n = 8)[a]

Treatment[b]	Nonacid-shock	Acid-shock	Acid-sensitive[c]	Acid-resistant, %
LE	5.64	2.36	2.84	0.052
HE	6.10	1.52	3.68*	0.003
RSH	5.73	1.57	3.37	0.007
ASH[d]	6.41	2.85	3.30	0.028
AG	6.01	2.78	2.78	0.059

[a]Maximal SEM of nonacid-shock and acid-shock concentrations was 0.401.
[b]LE = 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1}; HE = 0.215 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1}; RSH = 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$· d^{-1} with ruminal infusion of starch hydrolysate (SH); ASH = 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with abomasal infusion of SH; AG = 0.163 Mcal ME·(kg $BW^{0.75}$)$^{-1}$·d^{-1} with abomasal infusion of glucose.
[c]Probability of a larger variance ratio statistic; *$P < 0.05$.
[d]n = 7.

exposure (acid shock) were due to an increased level of acid production in the colon. In apparent contrast, Hovde et al. (1999) observed that *E. coli* O157:H7 was shed for longer periods of time in roughage- vs concentrate-fed cattle in experiments with steers given oral doses of *E. coli* O157:H7. Hovde et al. (1999) also noted differences in the acid resistance of coliforms between hay- and grain-fed steers, but the differences were much less dramatic (50 vs 86% survival for hay- and grain-fed animals, respectively) than those reported by Diez-Gonzalez et al. (1998). However, Tkalcic et al. (2000) inoculated calves with *E. coli* O157:H7 and observed no differences in fecal shedding of *E. coli* O157:H7 between calves that were fed roughage or concentrate diets. Tkalcic et al. (2000) also ran in vitro incubations of *E. coli* O157:H7 in rumen fluid from hay- and concentrate-fed steers and observed a rapid induction of acid resistance in rumen fluid from concentrate-fed steers.

In the present study, the fecal *E. coli* population in all steers appeared to be highly acid-sensitive. In all treatments, less than 1% of the *E. coli* survived when exposed to an acid environment (pH 2) for 1 h (Table 7). This is contradictory to what one would expect based on previous reports (Diez-Gonzalez et al., 1998; Hovde et al., 1999; Tkalcic et al., 2000) and considering that cecal and fecal pH ranged from neutral in the noninfused animals to as low as 5.2 in the infused animals (Table 2). It appears that tract pH alone is not a good indicator of the potential for wild-type *E. coli* to resist an acidic environment. The presence of VFA has been shown to be inhibitory for *E. coli* proliferation in the rumen (Wolin, 1969; Wallace et al., 1989; Rasmussen et al., 1993). Diez-Gonzalez and Russell (1999) demonstrated a strong correlation between the extreme acid resistance of *E. coli* O157:H7 and the concentration of undissociated VFA, and demonstrated that pH itself is not the inducer of acid-resistance properties. Consistent with our findings, they suggested that stressors other than pH could induce acid resistance in *E. coli.*

Simplistic predictions of pathogenic *E. coli* shedding based solely on the energy density of the diet or the location of carbohydrate digestion are unlikely to be accurate. A deeper understanding of the microbial dynamics in the gastrointestinal tract is needed to allow accurate prediction of changes in *E. coli* concentrations, specifically the pathogenic strains.

Implications

Increasing the energy supplied to the large intestine reduced pH and, therefore, altered the microbial profile. Changes in the total anaerobic and aerobic populations were observed; however, there was no affect on the concentration of fecal coliforms or *E. coli.* Fecal *E. coli* were highly acid-sensitive, even when fecal pH was neutral. More information is needed in order to accurately predict the response of specific species (i.e., pathogenic *E. coli*) to dietary changes.

Literature Cited

Allison, M. J. 1984. Microbiology of the rumen and small and large intestines. Page 340 in Dukes' Physiology of Domestic Animals. 10th ed. M. J. Swenson, ed. Cornell University Press, Ithaca, NY.

Allison, M. J., I. M. Robinson, R. W. Dougherty, and J. A. Bucklin. 1975. Grain overload in cattle and sheep: Changes in microbial populations in the cecum and rumen. Am. J. Vet. Res. 36:181–185.

Bauer, M. L., D. L. Harmon, D. W. Bohnert, A. F. Branco, and G. B. Huntington. 2001. Influence of alpha-linked glucose on sodium-glucose cotransport activity along the small intestine in cattle. J. Anim. Sci. 79:1917–1924.

Branco, A. F., D. L. Harmon, D. W. Bohnert, B. T. Larson, and M. L. Bauer. 1999. Estimating true digestibility of nonstructural carbohydrates in the small intestine of steers. J. Anim. Sci. 77:1889–1895.

Bryant, M. P., and I. M. Robinson. 1961. An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446–1456.

Diez-Gonzalez, F., T. R. Callaway, M. G. Kizoulis, and J. B. Russell. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science (Wash. DC) 281:1666–1668.

Diez-Gonzalez, F., and J. B. Russell. 1999. Factors affecting the extreme acid resistance of *Escherichia coli* O157:H7. Food Microbiol. 16:367–374.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA 97:2999–3003.

Harmon, D. L., and K. R. McLeod. 2001. Glucose uptake and regulation by intestinal tissues: Implications and whole-body energetics. J. Anim. Sci. 79(E. Suppl):E59–E72.

Hovde, C. J., P. R. Austin, K. A. Cloud, C. J. Williams, and C. W. Hunt. 1999. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. Appl. Environ. Microbiol. 65:3233–3235.

Mertens, D. R. 1983. Using neutral detergent fiber to formulate dairy rations and estimate their net energy content of forages. Pages 60–68 in Proc. Cornell Nutr. Conf. Ithaca, NY.

Rasmussen, M. A., W. C. Cray Jr., T. A. Casey, and S. C. Whipp. 1993. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli.* FEMS Lett. 114:79–84.

Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79:1503–1509.

Siciliano-Jones, J., and M. R. Murphy. 1989. Nutrient digestion in the large intestine as influenced by forage to concentrate ratio and forage physical form. J. Dairy Sci. 72:471–484.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. 7th ed. Iowa State Univ. Press, Ames.

Tkalcic, S., C. A. Brown, B. G. Harmon, A. V. Jain, E. P. Mueller, A. Parks, K. L. Jacobsen, S. A. Martin, T. Zhao, and M. P. Doyle. 2000. Effects of diet on rumen proliferation and fecal shedding of *Escherichia coli* O157:H7 in calves. J. Food Prot. 63:1630–1636.

USDA-APHIS. 1997. An update: *Escherichia coli* O157:H7 in humans and cattle. Centers for Epidemiology and Animal Health, Fort Collins, CO.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell Univ. Press, Ithaca, NY.

Wallace, R. J., M. L. Falconer, and P. K. Bhargava. 1989. Toxicity of volatile fatty acids at rumen pH prevents enrichment of *Escherichia coli* by sorbitol in rumen contents. Curr. Microbiol. 19:277–281.

Weimer, P. J. 1996. Why don't ruminal bacteria digest cellulose faster? J. Dairy Sci. 79:1496–1502.

Wolin, M. J. 1969. Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. Appl. Microbiol. 17:83–87.